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CONTENTS:

A COMPARISON OF THE ANTIGENIC AND CULTURAL CHARACTERISTICS OF A NUMBER OF STRAINS OF BACILLUS TYPHOSUS.
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A Comparison of the Antigenic and Cultural Characteristics of a Number of Strains of *Bacillus Typhosus*.*

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ALTHOUGH it has seemed to be the general concensus of opinion that *Bacillus typhosus* is a very homogeneous organism, yet in view of the fact that some observers have reported cultural and serological variations, it was thought advisable to investigate the cultural and serological reactions of the strains of *typhosus* used in this laboratory.

The work done may be divided into three phases, namely: cultural reactions, agglutination and absorption tests, and the Widal reaction. The source, place of isolation, name and date of the organisms used are tabulated in table I.

CULTURAL REACTIONS.

TECHNIQUE: The carbohydrate medium used was semisolid, to which was added 1 per cent of the carbohydrate desired, and Andrade indicator to make a pale, flesh color when cold. As a check a second set of determinations was run, using meat infusion broth adjusted to Ph, 7.0, to which 1 per cent of the carbohydrate was added, litmus being used as an indicator. For the lead acetate agar 1 per cent lead acetate solution was added to semisolid medium. Two per cent peptone gelatine, made according to a formula devised by Treece (1), was used for liquefaction and to test for gas production in noncarbohydrate media.

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TABLE I.—Organisms used for cultural and antigenic reactions.

No.	Source.	Name.	Date.
1	Blood culture—Lawrence, Kan.	57	1913
21	Blood culture—Kansas City, Mo.		1919
223	Blood culture—University of California.		1914
25	Blood culture—Johns Hopkins Hospital.		
33	Blood culture—Youngstown Hospital.	McCreary	1921
4	Feces—Lawrence, Kan.	Smith	1919
6	Feces—Lawrence, Kan.		1918
8	Feces—Lawrence, Kan.		1919
16	Feces—Carrier, Beau Desert, France.	Schopinsky	1918
20	Feces—Topeka, Kan.		1919
24	Feces—Fatal case, John Hopkins.		1919
27	Feces—Kansas City, Mo.		1920
28	Feces—Carrier.	Light	1920
29	Feces—Carrier.	Blythe	1920
30	Feces—Carrier.	Dardrich	1920
31	Feces—Carrier.	Cattler	1920
32	Feces—Carrier.	Doud	1920
34	Feces—Carrier.	Stitt	1920
35	Feces—Case.	Levi	1920
7	Spinal fluid—Halstead, Kan.		1919
12	Spleen—Autopsy.	Rawlings	
	Spleen—Autopsy.	Rawlings	
15	Gall bladder—Autopsy, France.	Wable	1918
2	No history—New York board of health.		
3	No history—New York city board of health.	Bender	
10		Mt. Sinai	
11	No history—New York city board of health.	Pfeiffer	
13	No history—American Museum.	Hopkins	
14	No history—American Museum.	Miller	
17	No history—Institute of Berlin.	Ebert	1888
19	No history—University of Chicago.	Jordan	1889
26	No history—Johns Hopkins Hospital.		

TABLE II.—Cultural characteristics.

Number.	Motility	Gram	Gelatine	Indol	Lead acetate	Dextrose	Mannite	Maltose	Lactose	Saccharose	Dextrin	Rhamnolitinose	Salacin	Dulcite	Arabinose	Xylose	Litmus milk.
1, 2, 3, 4, 6, 7, 8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	1 Neutral after 1 week.
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
10, 11, 13, 14, 15, 16, 17, 20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15, 17, 19, neutral after 3 weeks.
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
21	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	10 days.
23-25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	23, 24, 26, neutral after 3 weeks.
24, 28, 29, 30, 31, 32, 33, 34, 35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
26, 27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

The litmus milk was kept for six weeks before being discarded. The cultural reactions are tabulated in table II. It will be observed from the table that none of the strains exhibited any variation in the media commonly used in routine laboratory procedure. All strains gave acid in dextrose, mannite, maltose, negative in lactose and saccharose, no liquefaction of gelatine, no indol, and an initial acidity in litmus milk. Three strains gave slight acidity in salacin, one strain gave no acid in xylose, Rawlings' strain, and one gave acid only after ten days. Two strains were negative in dextrin. All strains except No. 7 gave a distinct greenish-black cloud around the stab in 2 per cent peptone gelatine, but no gas. In litmus milk all but six organisms remained a permanent lilac color, six turned back to neutral in three weeks and one became a deep blue after one week.

In addition to the above strains an organism isolated from the feces of a clinical case of mild typhoid was studied. This organism is designated as No. 5. The patient at no time gave a positive Widal. The organisms were abundant in the feces and culturally differed from *Bacillus typhosus* only in giving very slow blackening of lead acetate agar, negative in xylose, negative in dextrin, positive in rhamnose, and distinct alkaline reaction in litmus milk after 72 hours, but with no saponification.

DISCUSSION.

Weiss (2) has reported the cultural characteristics of thirty-one strains of *typhosus* and groups them according to xylose fermentation. Three of his strains produced acid slowly and four remained negative. One of the negative strains was the Rawlings' strain which we also found to be negative.

Teague (3) objects to such a classification on the basis of xylose fermentation on the ground that the so-called negative strains are not really incapable of fermenting xylose, but ferment it slowly. Four of his strains failed to give acid on the thirty-second day, but these strains could be trained to give acid by plating on xylose agar. No attempt was made by the author to discover mutants from negative strains on any of the carbohydrates used.

Our strains were uniformly negative on dulcite and arabinose. Teague (3) reports eleven out of forty-one strains fermenting these sugars slowly. Krumwiede (4) also reports the fermentation in dextrin as varying with the sample used. The two cultures giving negative in dextrin might, therefore, have shown typical acid production with another sample.

The salacin fermentation seemed variable and did not correlate with any other characteristics.

The danger of confusing nongas-producing paratyphoid strains with *typhosus* has been recently emphasized. Ten Broek (5) reports a nongas-producing hog-cholera bacillus which resembles in some respects *B. typhosus*. Krumwiede (4) also reports a similarity both culturally and serologically between *B. pullorum* and *B. sanguinorum* and *B. typhosus*. Myers (6) reports the isolation of a rhamnose positive *typhosus* from a clinical case of typhoid which was also atypical in its serological reaction. It was difficult to decide, therefore, whether No. 5 was a true but irregular typhoid or a nongas-producing paratyphoid. Krumwiede (7), using the fermentation of rhamnose as the deciding factor between typhoid and paratyphoid, would place it in the para group.

AGGLUTINATION AND ABSORPTION TESTS.

Antigenic irregularities had been observed in this laboratory in the course of routine agglutination tests on organisms isolated from clinical cases of typhoid and a number of Widals. Parke-Davis antityphoid serum, serum from the city laboratory of Wichita, Kan., and serum sent us from the University of Chicago were used in checking up the antigenic properties of the following organisms: Nos. 1, 2, 4, 5, 20, 50, 51 and 52.

Culturally they were all typhoid. Nos. 50, 51 and 52 were strains isolated from feces in cases resembling influenza. They are not included in the other tables because of accidental loss.

TABLE III.—Quantitative variations in agglutinations with commercial sera.

No.	Sera used.					
	Parke-Davis.		Wichita.		University of Chicago.	
	Titre.	Reaction.	Titre.	Reaction.	Titre.	Reaction.
1	1-50	—	1-50	.....	1-8000	4+
2	1-10000	3+	1-50	1+	1-10000	4+
4	1-1000	4+	1-400	4+	1-2000	4+
6	1-2000	4+	1-400	.....	1-4000	4+
50	1-50	—	1-50	.....	1-50	.....
51	1-50	—	1-50	.....	1-50	.....
52	1-50	.....	1-50	.....	1-50	.....
20	1-4000	4+	.....	.....	1-8000	4+

Numerous observers have remarked on the antigenic differences in typhoid. Durham (8) observed such differences, but did not attempt to group his strains. Weiss (1) and Hooker (9), however, offered a tentative grouping on the basis of their agglutination and absorption tests.

The agglutination tests in this series were all done with suspensions in sterile saline made from twenty-four-hour cultures. The serum used came principally from rabbits immunized in this laboratory.

A high-titred bivalent horse serum from the New York city board of health* prepared from the Mt. Sinai strain, and a freshly isolated strain as well as a high-titred serum for which the Rawlings strain had been used for immunization from the Lederle laboratories, were also used. Table IV gives a summary of the results. In addition to the results given here, eight other immune sera were used for agglutination against all the organisms with similar results.

The following technique was used for the absorption tests: The serum to be tested was diluted to one-tenth of the titre. This dilution was then saturated with organisms, washed from a twenty-four-hour agar slant to make a heavy emulsion. This was incubated at 37° C. for four hours and for four days at ice-box temperature, more organisms being added as the supernatant fluid became clear. The control of diluted serum in every case gave a good agglutination in spite of the prolonged incubation. If the control gave agglutination after absorption with the homologous organism the test was repeated.

Since considerable prominence has been given to the mirror reaction in the recent literature, it might be well to establish some standard method for absorption tests in order to get comparable results. We found the following points must be carefully considered in any test:

1. Weight of suspension.
2. Dilution of serum.
3. Time of absorption.
4. Repeated saturation.
5. Temperature.
6. Controls.

Krumwiede (4) recommends a proportion of 1-4 or 3, or at most 1-2 of packed cells to supernatant fluid. Our proportion after the final centrifugation was about 1-3. It was found that a dilution of one-tenth the titre of the serum was perfectly satisfactory. Although higher dilutions could be used, a lower dilution did not give complete absorption. Three or four hours was not long enough

*I am indebted to the kindness of Dr. Charles Krumwiede for the use of this serum.

to give complete absorption and frequently absorption was not complete in twenty-four or forty-eight hours. After a standard of four days was chosen no more trouble was experienced. It was always necessary to add more organisms as the supernatant fluid became clear; the greater the tendency to agglutinate, the larger the number of organisms necessary for complete absorption. It was necessary to keep the serum at ice-box temperature because of the well-known tendency of diluted serum to deteriorate at room or incubator temperatures. A control of diluted serum which had been incubated under the same conditions as the test sera was necessary to show that no drop in titre had occurred, and a control of the serum to be tested saturated with the homologous organisms indicated the completeness of the absorption. Table V gives a summary of the absorption tests.

From table IV it will be seen that the strains of typhoid differ perceptibly in their agglutinating properties. On this basis we have placed the organisms tentatively into three groups. Group I is made up of eleven organisms; group II of twelve organisms; group III of two organisms. Group I serum agglutinates all other organisms in this group in dilutions practically as high as that given for the homologous organisms. Group I serum also agglutinates group II organisms, but in lower dilutions; conversely, the group I organisms are agglutinated by group II serum, but in lower dilutions than are the group II organisms. These two groups are closely related and interagglutinate to the degree indicated in the table. Groups I and II serum give slight or no agglutination with group III organisms. Group III, consisting of two strains, Nos. 2 and 3, interagglutinate perfectly at 1-15000, but this high-titred serum agglutinates members of groups I and II in low dilutions or not at all.

The results of agglutination tests using horse serum indicated that the same antigenic differences were present, but that they appeared in higher dilutions because of the higher titre of the serum.

To illustrate: No. 12, the Rawlings strain, was completely agglutinated at 1-80000, and No. 1 at 1-5000.

Many of these agglutination tests were checked by using the microscopic method, care being used to rule out the personal equation. Where partial agglutination occurred, the macroscopic method seemed to give more definite results.

It will be seen that the absorption tests show an even closer relationship between groups I and II than do the agglutination tests, No. 1 being somewhat more irregular than the others. The ab-

TABLE IV.—Agglutination reactions with immune sera.

Strain.	Serum.													
	1		4		7		20		12—Lederle.		2		3	
	Titre.	Reaction.	Titre.	Reaction.	Titre.	Reaction.	Titre.	Reaction.	Titre.	Reaction.	Titre.	Reaction.	Titre.	Reaction.
1	1–2000	.....	1–1000	.....	1–500	3+	1–2000	4+	1–2000	3+	1–100	4+	1–100	+
4	1–2000	.....	1–1000	.....	1–500	4+	1–4000	3+	1–2000	4+	1–100	+	1–100	±
6	1–2000	.....	1–500	.....	1–500	5+	1–2000	3+	1–5000	3+	1–100	±	1–100	±
7	1–1000	.....	1–1000	.....	1–3000	4+	1–3000	3+	1–8000	3+	1–100	2+	1–100	3+
8	1–200	.....	1–500	.....	1–3000	4+	1–2000	3+	1–2000	3+	.....	.....	.....	.....
20	1–1000	.....	1–1000	.....	1–500	3+	1–3000	4+	1–2000	3+	.....	.....	.....	.....
21	1–1000	.....	1–500	.....	1–2000	3+	1–2000	3+	1–2000	3+	.....	.....	.....	.....
23	1–200	.....	1–500	.....	1–1000	3+	1–1000	3+	1–8000	3+	.....	.....	1–200	3+
24	1–1000	.....	1–500	.....	1–3000	3+	1–3000	4+	1–8000	4+	.....	.....	1–100	2+
25	1–50	.....	1–1000	.....	1–1000	3+	1–3000	4+	1–5000	3+	.....	.....	1–200	3+
26	1–500	.....	1–2000	.....	1–3000	4+	1–3000	4+	.....	.....	.....	.....	.....	.....
27	.....	.....	.....	.....	1–1000	4+	.....	.....	1–1000	4+	.....	.....	.....	.....
28	.....	.....	.....	.....	1–1000	4+	1–1000	4+	1–5000	4+	.....	.....	1–50	3+
29	.....	.....	.....	.....	1–500	4+	1–1000	4+	1–5000	.....	.....	.....	1–50	2+
11	1–200	.....	1–500	.....	1–1000	3+	1–500	3+	1–8000	4+	1–200	4+	1–100	4+
9	1–2000	.....	1–2000	.....	1–2000	3+	1–3000	3+	1–5000	3+	1–1000	3+	1–1000	3+
12	1–1000	.....	1–2000	.....	1–2000	4+	1–2000	4+	1–8000	3+	.....	.....	.....	.....
10	1–1000	.....	1–2000	.....	1–500	3+	1–2000	3+	1–5000	3+	1–200	3+	1–1000	3+
13	1–1000	.....	1–1000	.....	1–200	3+	1–2000	3+	1–5000	4+	1–200	4+	1–200	3+
14	1–1000	.....	1–2000	.....	1–1000	3+	1–1000	3+	1–2000	3+	1–200	3+	1–1000	3+
15	1–1000	.....	1–500	.....	1–2000	3+	1–2000	3+	1–500	3+	1–100	3+	.....	.....
16	1–1000	.....	1–5000	.....	1–5000	3+	1–3000	3+	1–5000	4+	1–100	2+	1–100	4+
17	1–1000	.....	1–5000	.....	1–1000	3+	1–300	3+	1–5000	3+	1–100	2+	.....	.....
19	1–1000	.....	1–1000	.....	1–1000	3+	1–1000	3+	1–5000	4+	1–100	2+	1–100	±
30	.....	.....	.....	.....	1–1000	4+	1–1000	4+	1–8000	4+	1–50	3+	1–50	3+
31	.....	.....	.....	.....	1–100	4+	1–1000	4+	1–8000	4+	1–50	±	1–50	2+
32	.....	.....	.....	.....	.....	.....	1–100	4+	1–5000	3+	.....	.....	1–50	3+
2	1–200	.....	1–500	.....	±	.....	±	.....	1–50	3+	1–15000	4+	1–12000	3+
3	1–200	.....	.....	.....	±	.....	±	.....	1–50	3+	1–15000	3+	1–12000	4+

TABLE V.—Absorption tests with Immune sera.

Absorbing antigen.	Sera used.									
	1	12	9	2	3	27	7	13	20	8
1	+	±	±	±	—	±	±	+	±	±
4	+	+	+	—	—	+	+	+	+	+
6	±	+	±	—	—	—	+	+		+
7	±	+	+	—	±	+	+	+	+	+
8	±	+	+	—	—	—	+	+	+	+
20	±	±	+		—	+	+	+	+	+
21	+	+	±	—	—	+	+	+	+	+
23	+	±	+	±	±	+	+	+	+	+
24	+	+	+	—	—	+	+	+	+	+
25	+	±	+	—	—	+	+	+	+	+
26	+	+	±	—	—	+	+	+	+	+
27	+	—	+	—	—	+		+	+	+
9	+	+	+	—	—	+	+	+	+	
10	+	+	+	—	—	+	—	±	+	±
11	±	+	+	—	—	+	—	+	—	—
12	±	+	+	±	—	±	—	+	+	+
13	+	+	+	—	—	±	±	+	±	+
14	+	+	±	+	+	±		+	+	+
15	±	—	+	—		±	+	+	+	+
16	+	+	+	—		+	+	+	+	+
17	+	±	+	±	—	+	+	+	—	+
19	+	+	+	—		+	+	+	+	+
2	—	±	—	+	+	—	—	—	—	—
3	—	±	—	+	+	—	—	—	—	—

+ Absorption complete.
± Absorption incomplete but reduction of titre.
— No absorption.

sorption tests show a more striking difference between the two organisms in group III and the other groups. The antigenic differences shown by these organisms could not be correlated with their age as with Hooker's (9) organisms, nor with cultural differences as with Weiss' (2).

No. 5 was found to be entirely inagglutinable by any of the sera used. Serum prepared from this organism agglutinated only the homologous organism. It did not absorb any of the agglutinins from the sera prepared from other organisms, nor were its agglutinins absorbed by other organisms. These facts, in connection with the somewhat irregular carbohydrate reactions and the atypical

growth on agar slants, made it seem advisable to consider this organism one of those unclassified, irregular organisms which are not infrequently isolated from stools, although in many respects this does not differ any more radically than irregular strains reported by other observers.

In running Widals in this laboratory it was customary to set up each serum with *B. typhosus,* para A and para B. A member of the department suggested that it might be advisable to use several strains of *B. typhosus* in setting up routine Widals. Accordingly, a Widal giving negative with the strain used, No. 2, was again set up, using three other strains of typhoid. It again gave a negative with No. 2, but was strongly positive with the other two strains. It was recognized that apparent antigenic differences of this sort might constitute an important source of error in making routine laboratory tests.

The sera for the Widals were obtained from various sources. Sera A, C, D, F, G, J and I were from clinical cases of typhoid from which the organism was subsequently isolated. The others came as positive Widals from reputable laboratories, the majority of which use the Rawlings strain. Most of the specimens were drops of blood dried on a metal slide or on filter paper. A dilution of 1–25 and 1–50 was made and an equal amount of a living suspension of the organism was added, making an ultimate dilution of 1–50 and 1–100. All Widals were set up using Nos. 1, 2, 3, 10 and 12. No. 12 was selected because it is the Rawlings strain and is used for the army vaccine. Numbers 2 and 3 were used because of the irregularities exhibited in the absorption tests and No. 10 because it was an organism giving a clear adherent agglutination with most sera used. The results of these tests may be seen in table VI.

It was noticed that fresh serum drawn from the clot and used within twenty-four or forty-eight hours gave positive agglutination with a larger number of organisms than those made from dried blood. In those Widals run with dried blood precipitation was usually marked in the tubes giving a positive Widal. This might be due to the presence of hemoglobin, foreign substances on the metal slides or paper, some change in reaction, or some biochemical change. This phenomenon is being investigated. No precipitation was noted in the Widals using clear serum, nor in the agglutination tests with rabbit serum. Stober (14) mentions the occurrence of both precipitation and agglutination with his immune sera.

TABLE VI.—Widal reactions.

Organisms.	Sera.																			Percentages.		
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	+	±	—
1.....	+	+	+	+	+	+	|	+	+	+	+	|	±	+	+	±	±	+	+	+	42	10	47
2.....	|	±	+	|	|	±	±	|	+	|	±	|	|	+	+	±	|	|	20	25	55
3.....	|	|	+	±	±	±	+	|	+	|	|	+	+	±	+	+	±	|	|	33	27	38
10.....	+	.....	+	+	+	+	+	.....	+	.....	±	±	±	+	|	±	+	+	+	+	+	70	23	6
12.....	+	+	+	+	+	+	|	+	+	+	+	|	±	+	+	+	+	+	+	+	75	15	10

+ = Clear-cut complete agglutination.
± = Partial agglutination.
— = Negative agglutination.

From table VI it is readily seen that different organisms with the same sera set up at the time showed marked differences in agglutinability. This may be due to the different agglutinabilities inherent in the organisms themselves and such marked differences probably would not be noted had absorption tests been possible. It is recognized that these twenty positive Widals are too few to provide a basis for accurate conclusions. It seems highly probable that the dried-blood method exaggerates the antigenic differences between the organisms, changing what is probably a quantitative into an apparently qualitative difference between the organisms. The low percentage of positives given with Nos. 2 and 3 might be expected from the results given in the absorption tests using immune sera. No. 10, on the other hand, gave a very low percentage of negatives. Those read as partial agglutination in clinical work would be called positive. The tubes read as positive gave complete clearing of the supernatant fluid; those read as partial agglutination showed unmistakable agglutination, but with some cloudiness of the supernatant fluid. No. 10, therefore, gave 93 per cent positive. No. 12, while giving the highest percentage of complete agglutinations, gave only 90 per cent positive when partial agglutinations are included. It seems probable in view of the results obtained that it might be worth while to use more than one strain of typhoid in running Widals and to select easily agglutinable strains, such as No. 10 Mt. Sinai strain, and No. 12 the Rawlings strain.

The serological reactions here recorded might have an important bearing on the following points:

1. The occurrence of typhoid fever in vaccinated persons.
2. The advisability of using a polyvalent vaccine.
3. The occurrence of negative Widals in clinical cases of typhoid fever.
4. Sources of error due to the dried-blood method.

A number of cases of typhoid fever occurring in vaccinated individuals may be found in the literature. Vaughn (10) says that "It is possible that in so far as vaccination has failed it is due to the disease being caused by other members of the typhoid group, . . . which in all probability is much larger than we now appreciate." Mock (11) reports the occurrence of forty-five cases of typhoid and paratyphoid in individuals who had been vaccinated about one year previous to the attack. Some of the strains isolated were atypical in regard to their cultural and serological reactions, but were identified positively as typhoid or paratyphoid organisms.

Trowbridge (12) reports the occurrence of a typhoid epidemic among vaccinated persons in an institution. Here the original source of infection came from the milk supply, which was infected by a vaccinated worker with a mild case of typhoid. It is realized that in such an epidemic the dosage may have been sufficient to overcome the immunity acquired from vaccination. Wade and McDaniel (13) report the occurrence of an epidemic in an institution among vaccinated individuals. Here there seemed to be an interesting correlation between the negative Widals given after vaccination and the susceptibility of these persons to typhoid. Myers and Nielson (6) report the isolation of an atypical strain of typhoid from the blood stream and stool, respectively, of two vaccinated persons.

Hooker (9) and Weiss (2) conclude from their experiments that a vaccine made from several strains of typhoid would be more efficient than one made from a single strain. The results of these observers and the others reported, together with our findings, would suggest that at least it might be well to consider the use of a vaccine made from several strains.

Stober (14) reports three negative Widals and seven positive Widals, using an organism isolated from urine. Mock (11) also reports negative agglutination with typical typhoid organisms isolated from clinical cases. Robinson (15), on the other hand, reports no variability in 100 Widals using the Worcester and Rawlings strains.

In summing up the work done the following conclusions may be drawn:

1. Culturally, the typhoid organisms studied differ very slightly from each other, the reaction being most variable in dextrine, xylose, salacin and litmus milk. These variations cannot be correlated with the age of the culture nor source.

2. Cross-agglutination and absorption tests establish the existence of at least quantitative antigenic differences between the strains used. It occurs to the author that the conflict as to whether there are antigenic differences in the typhoid group may be due to the fact that qualitative rather than quantitative differences have been emphasized.

3. There is a marked difference in the agglutination of organisms with the sera used in Widals, and it would be advisable to set up each Widal with more than one strain, selecting strains which were known to give a high percentage of positives.

4. The use of fresh serum drawn from the clot is much more satisfactory than the use of dried blood, changing what is probably a quantitative difference into an apparently qualitative difference.

This work was offered as part of the requirement for a master's thesis.

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